Investigation of Pyrene Degradation and Bound Residue Formation on Environmental Surfaces

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are one of the primary contaminants of concern at the Champion International Superfund Site in Libby, Montana. Contaminated soil at the Libby site is currently being treated by bioremediation that results in contaminant loss mechanisms including mineralization and formation of bound residues (Nieman et al., 1999). Our current work involves the use of synchrotron based FTIR spectromicroscopy at the Lawrence Berkeley National Laboratory (LBNL) Advanced Light Source (ALS) beamline 1.4.3 to observe pyrene degradation in model environmental systems. This instrument was recently used to monitor bacterial chromium reduction on a basalt surface (Holman et al., 1999). Our research team used synchrotron based FTIR spectromicroscopy to characterize the degradation of pyrene on a magnetite surface by three bacterial strains isolated from contaminated soil from the Champion International Superfund site in Libby, Montana. The three strains were characterized as *Mycobacterium* Spp. by grams stain, 16S ribosomal RNA, and fatty acid analyses. They were isolated from soil samples from the Libby site, are capable of ^{14}C-pyrene degradation, and were shown to metabolize ^{14}C-pyrene to $^{14}CO_2$ in the absence of other carbon sources (unpublished data from UWRL).

RESULTS

When introduced onto a pyrene coated magnetite surface, the synchrotron-based FTIR spectra showed that the three strains were capable of pyrene degradation. One strain proliferated and spread on the surface, forming a geometrically amorphous biofilm. The corresponding spectral markers further showed a reduction of pyrene and the presence of phthalic acid, a breakdown product of pyrene, on surfaces occupied by the bacteria. The other strain did not proliferate like its counterpart. Instead it formed small (<50 micron) but distinct colonies on part of the surfaces. Neither pyrene nor the three readily obtainable metabolites, phthalic acid, cinnamic acid, or pyrenol were detected on surfaces occupied by these colonies. Growth of the third strain was very slow, but disappearance of pyrene was observed after several months.

Current work involves the observation of pyrene degradation on the surface of a more complex humic acid surface. This work involves the assessment of the use of FTIR spectromicroscopy to detect the presence of humic acid, pyrene, and bacteria in a single sample so that degradation may be monitored over time. The FTIR spectrum of humic acid (soil humic acid standard from the International Humic Substance Society) obtained using FTIR spectromicroscopy at LBNL is shown in Figure 1. Figure 2 shows spectrum of the same humic acid after pyrene and pyrene degrading bacteria have been added to the system. The presence of bacteria is indicated by bacterial amide peaks at 1652 and 1541 wavenumbers. Humic acid is indicated by the peak at 1397 wavenumbers and the presence of pyrene is indicated by the three peaks at 831, 745, and 703 wavenumbers caused by out of plane hydrogen bending in the pyrene molecule.

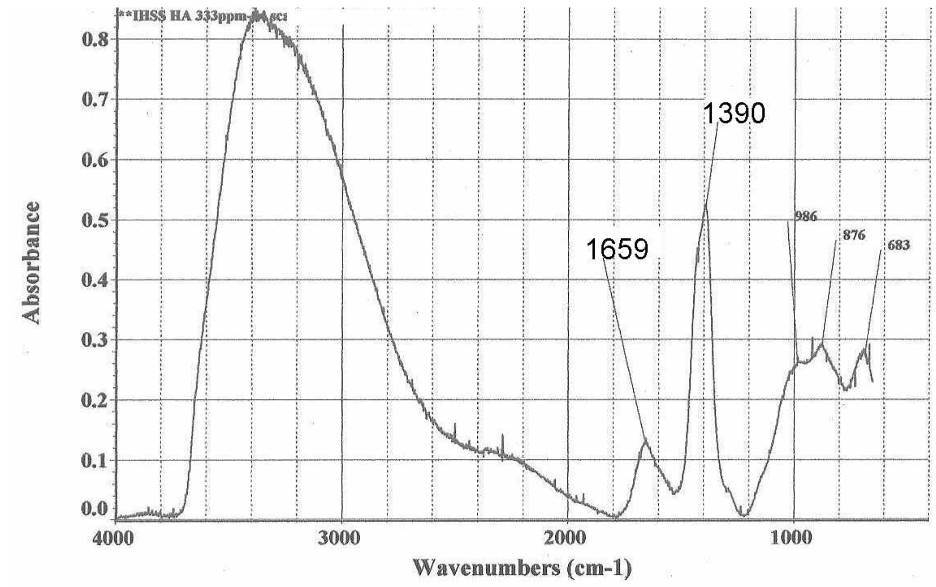

Figure 1. FTIR spectrum of soil humic acid standard (obtained from the International Humic Substances Society) produced by synchrotron-based FTIR spectromicroscopy analysis.

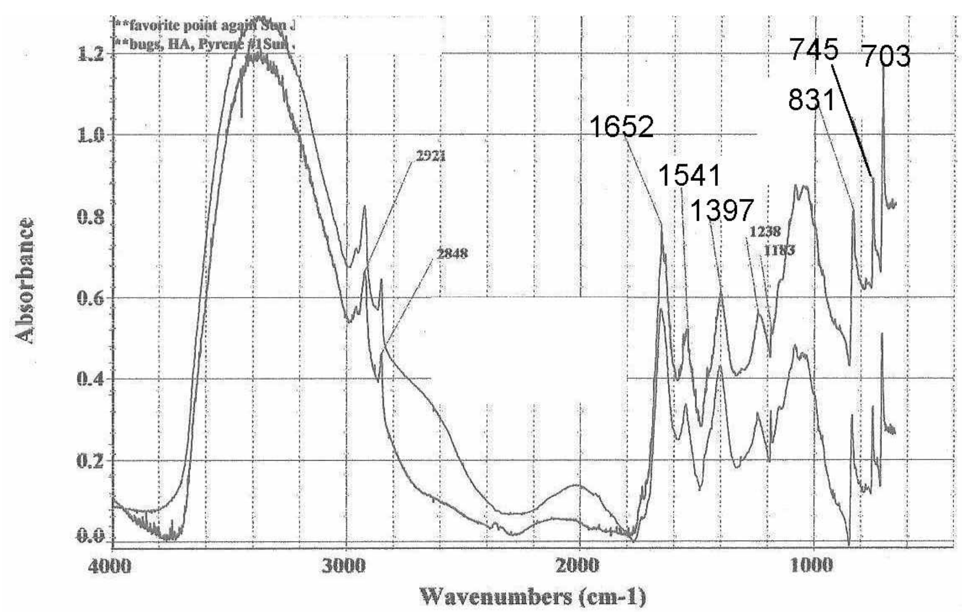

Figure 2. Synchrotron-based FTIR spectromicroscopy spectra of soil humic acid standard after pyrene and pyrene degrading bacteria have been applied to the humic acid surface. Time difference between acquisition of the two shown spectra of the same sample area is 6 hours.

By using the appropriate peaks to indicate the presence of a given component, surface area maps can be produced to show the two dimensional distribution of bacteria, humic acid, and pyrene on the sample surface. This distribution can then be monitored over time to detect contaminant degradation or transformation in situ, something that can not be accomplished using traditional treatability study techniques that involve the use of chemical extractants or costly radiolabeled compounds. The ability of the synchrotron-based FTIR microscope to focus on areas as small as 10μm allows for mapping at much higher resolution than standard FTIR microscopes. Future research will involve the study of potential changes in humic acid composition due to the microbially mediated incorporation of bound residues during the bioremediation process.

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